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The atherosclerotic plaque is a complex environment containing immune and stromal cells, many switching phenotype or even transitioning to alternative cell types [1–4]. Previous attempts to characterize heterogeneity in atherosclerosis have been limited by the requirement for predefined cell surface markers, thus negating new population discovery [5▪]. Consequently, cellular heterogeneity is poorly characterized and likely oversimplified [6–8].

This challenge in the atherosclerosis field has recently been addressed by the utility of single cell RNA sequencing (scRNA-seq), allowing transcriptome analysis at a single cell resolution [9▪▪,10▪▪]. In contrast to previous priori-dependant approaches, scRNA-seq allows for the unbiased, comprehensive characterization of cellular identity within a heterogeneous population [5▪]. Importantly, droplet-based microfluidic platforms permit the sequencing of thousands of cells in parallel [11].

Two recent studies have utilized a droplet-based scRNA-seq platform to map leukocyte heterogeneity in control and fat-fed low-density lipoprotein receptor deficient ($Ldlr^{-/-}$) [9▪▪] or apolipoprotein E ($Apoe^{-/-}$) mice [10▪▪], respectively. Unsupervised clustering revealed more than 10 clusters in each model, with increased heterogeneity with advancing disease. Both publications show that the atherosclerotic plaques were predominantly composed of macrophages and T cells. Macrophages were found to account for a lower proportion of aortic leukocytes in $Apoe^{-/-}$ mice compared to that observed in $Ldlr^{-/-}$ mice, although the proportion of macrophages increased with disease progression in both models [9▪▪,10▪▪].

Importantly, scRNA-seq identified a new macrophage cluster (identified by enriched expression of TREM2) and changed pervading views on macrophage biology, as the identified inflammatory and resident macrophage populations demonstrated only a weak resemblance to polarized M1 and M2 macrophage phenotypes, again highlighting the limitations of the current classification system [10▪▪].

Understandably, the authors initially employed this new technology using homogenous mouse models, but it will be clearly important to utilize this technology in human vessels with underlying atherosclerosis where heterogeneity will be greater and more difficult to clearly quantify. Owing to changes in cell phenotype occurring asynchronously, the transcriptional signatures of transitioning cells can be used to reconstruct the associated trajectory [12]. This technology, termed as pseudotime analysis, has previously been utilized to identify potential regulators of myeloid and lymphocyte differentiation [13,14]. Consequently, application of pseudotime analysis may be crucial to identifying putative regulators of important dynamic processes such as the transdifferentiation of smooth muscle cells to macrophage-like cells [1,2]. Additionally, using an in-silico receptor-ligand screen such as that used by Camp et al. [15] could provide insight into the level of crosstalk between cell types, thus providing further insight into the pathogenesis of atherosclerosis. Together, single cell technology and advanced computational analyses will provide exciting opportunities to exploit cellular heterogeneity in the cardiovascular system, which will aid in understanding the healthy and disease vessel wall cellular and molecular dynamics, as well as refining future therapeutic and diagnostic strategies. However, complete characterization of atherosclerosis is likely to require the advent of new technologies capable of integrating the transcriptional characterization of single cells with genomic, epigenetic, and spatial proteomic data.

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Conflicts of interest

There are no conflicts of interest.

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